Effect of Arginine Vasopressin on the Canine Epicardial Coronary Artery. Experiments on V_1-Receptor-Mediated Production of Nitric Oxide

Paulo Roberto B. Evora, Paul J. Pearson, Alfredo J. Rodrigues, Fernanda Viaro, Hartzell V. Schaff

Rochester, MN, USA – Ribeirão Preto, SP - Brazil

Objective - *To determine whether arginine vasopressin releases endothelium-derived nitric oxide (EDNO) from the epicardial coronary artery.*

Methods - *We studied segments of canine left circumflex coronary arteries suspended in organ chambers to measure isometric force. The coronary artery segments were contracted with prostaglandin $F_{2\alpha}$ (2×10^{-6}M) and exposed to a unique, strong arginine vasopressin concentration (10^{-6}M) or titrated concentrations (10^{-9} a 10^{-5} M).*

Results - *The unique dose of arginine vasopressin concentration (10^{-6}M) induced transient, but significant ($p<0.05$), relaxation in arterial segments with endothelium, and an increase, not significant, in tension in arteries without endothelium. Endothelium-dependent relaxation to arginine vasopressin was inhibited by Ng-monomethyl-L-arginine (L-NMMA, 10^{-5}M) or N^G-nitro-L-arginine (L-NOARG) (10^{-4}M), 2 inhibitors of nitric oxide synthesis from L-arginine. Exogenous L-arginine (10^{-4}M), but not D-arginine (10^{-4}M), reversed the inhibitory effect of L-NMMA on vasopressin-mediated vasorelaxation. Endothelium dependent relaxation to vasopressin was also reversibly inhibited by the vasopressin V_1-receptor blocker d(CH_2)$_5$Try(Me) arginine vasopressin (10^{-6}M) ($n=6$, $P<0.05$).*

Conclusion - *Vasopressin acts through V_1 endothelial receptors to stimulate nitric oxide release from L-arginine.*

Keywords: coronary artery, vasopressin, endothelium, nitric oxide

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Mailing address: Paulo Roberto B. Evora - Rua Rui Barbosa, 367/15 – 14015-120 Ribeirão Preto, SP - Brazil - E-mail: prbevora@keynet.com.br

Arginine vasopressin is an endogenous vasoactive peptide that has heterogeneous vascular effects depending on arterial diameter [1], location [2,3], oxygen tension [4], and the presence of an intact endothelium [5]. Arginine vasopressin is released in response to hypotension [6], hypovolemia [7], and hypoxemia [8] and is elevated following cardiopulmonary bypass [9] and during evolving myocardial infarction [10,11].

Vasopressin has been reported both to dilate and constrict coronary arteries. In the coronary circulation, vasopressin mediates endothelium-dependent relaxation in isolated large coronary arteries in vitro [2]. In vivo, however, vasopressin causes modest vasodilatation (or no change) in coronary arteries greater than 90 μm in diameter, but it constricts smaller arteries and arterioles [1].

The purpose of this experiment was to confirm the action of arginine vasopressin on the epicardial coronary artery in vitro and to determine whether nitric oxide could be implicated in endothelium-dependent vasodilatation to vasopressin previously reported in the coronary artery [2]. Release of endothelium-derived nitric oxide (EDNO) from the epicardial coronary artery would be expected to decrease epicardial coronary vascular resistance [12] and inhibit platelet activation in the coronary circulation [13]. Such an effect would be beneficial in the downstream arterioles where vasopressin-mediated vasoconstriction would induce platelet activation secondary to increased shear forces in the constricted arterial beds.

Methods

Heartworm-free mongrel dogs (25-30 kg) of either sex were anesthetized with pentobarbital sodium (30 mg/kg intravenous injection; Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) and exsanguinated via the carotid arteries. The chest was quickly opened, and the heart was harvested and immersed in cool, oxygenated physiological salt solution of the following composition (mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.22, $CaCl_2$ 2.5, $NaHCO_3$ 25.0,

Ca-EDTA 0.016, and glucose 11.1 (control solution). The procedures and handling of the animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Mayo Foundation.

In vitro experiments - Canine left circumflex coronary arteries were dissected free of connective tissue and placed in the oxygenated physiological salt solution. Segments (4-5 mm in length) of blood vessel were prepared with special care not to touch the intimal surface. In some of the segments in which vascular smooth muscle function was to be tested without the influence of the endothelium, the intimal surface was removed by gently rubbing the inner surface of the blood vessel with a pair of watchmaker forceps. This procedure removes endothelium but does not affect the ability of vascular smooth muscle to contract or relax (fig. 1) [14].

Coronary artery segments, with and without endothelium, were suspended in organ chambers (25 mL) filled with control solution (physiological salt solution of the following composition (mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.22, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, and glucose 11.1), maintained at 37°C and bubbled with 95% O_2/5% CO_2 (pH = 7.4). Two stainless steel hooks passed through its lumen suspended in each ring. One clip was anchored to the bottom of the organ chamber, and the other was connected to a strain gauge for measurement of isometric force (Grass FTO3, Grass Instrument Company, Quincy, Massachusetts). The rings were placed at the optimal point of their length-tension relation by progressively stretching them until the basal applied tension was 10 grams. In all experiments, the presence or absence of endothelium was confirmed determining the relaxing response to acetylcholine (10^{-6} M) in rings contracted with potassium ions (20 mM) [14,15]. After optimal tension was achieved, the arterial segments were allowed to equilibrate for 30-45 minutes before administration of drugs. The time between the treatment with indomethacin, NO-synthase inhibitors, vasopressin antagonist, L-arginine, D-arginine, and the beginning of the prostaglandin $F_{2\alpha}$ contraction was also 30-45 minutes. The time between 2 different concentrations of arginine vasopressin during the concentration-response curve was around 3 minutes. After prostaglandin $F_{2\alpha}$ contraction was stable, 3 kinds of experiments were performed:

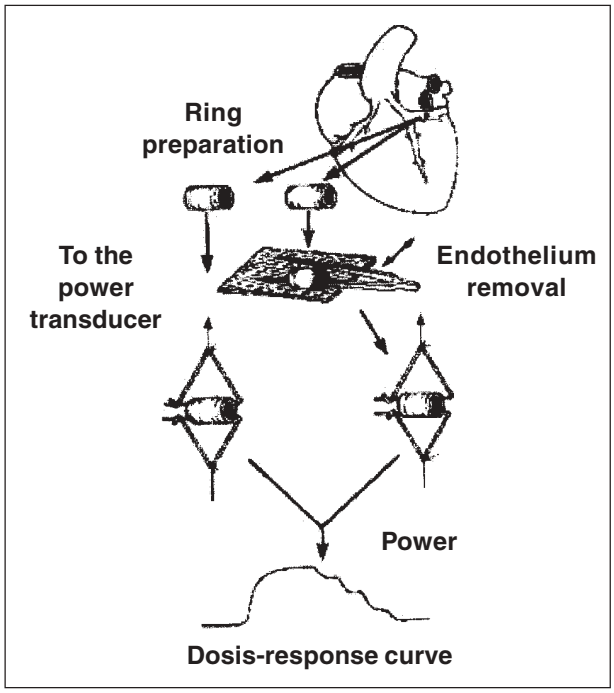

Fig. 1 - Methodology schematic representation.

Experiment 1 - Eighteen coronary pairs (with and without endothelium) were exposed to a single high concentration of vasopressin (10^{-6} M). Six pairs were incubated with indomethacin, and 12 pairs of coronary rings were incubated with nitric oxide synthesis inhibitors: 6 with L-NMMA (10^{-5} M) and 6 with L-NOARG (10^{-4} M).

Experiment 2 - Six pairs underwent vasopressin concentration-response curves (10^{-9} to 10^{-5} M) in the presence of indomethacin.

Experiment 3 - Six pairs of coronary rings were incubated with indomethacin and d(CH2)5Try(Me) arginine-vasopressin (10^{-6} M) and exposed to a single dose of vasopressin.

The nitric oxide synthesis inhibition was confirmed by using L-arginine (10^{-4} M) or D-arginine (10^{-4} M).

The following drugs were used: acetylcholine chloride, [Arg^8] vasopressin, acetate salt, [ß-mercapto-ß,ß-cyclopentamethylenepropionyl', -O-Me-Try^2, Arg]-vasopressin, indomethacin, prostaglandin $F_{2\alpha}$, (Sigma Chemical Company, St. Louis, Missouri), L-arginine, D-arginine, Ng-monomethyl-L-arginine, and N^G-nitro-L-arginine (Calbiochem, San Diego, California). All powdered drugs were prepared with distilled water except for indomethacin, which was dissolved in Na_2CO_3 (10^{-5} M). The concentrations are expressed as the final molar concentration in the organ chambers. To examine endothelium-dependent relaxation to [Arg^8] vasopressin, acetate salt, vascular segments were contracted with prostaglandin $F_{2\alpha}$ and then exposed to increasing concentrations of vasopressin.

Results are expressed as mean ± SEM. In all experiments, "n" refers to the number of animals from which blood vessels were taken. In segments contracted with prostaglandin $F_{2\alpha}$, responses are expressed as percent changes from the contracted levels. Statistical evaluation of data was performed with the Student t test for either paired or unpaired observations. Values were considered statistically significant when P was less than 0.05.

Results

Coronary artery segments with and without endothelium exhibited comparable contraction to prostaglandin $F_{2\alpha}$ (2×10^{-6}M), 5.05±0.86 and 4.72±1.80 grams, respectively, for arteries with and without endothelium (n = 6).

Experiment 1 - After contraction to prostaglandin was stable, administration of a high concentration of vasopres-

sin (10^{-6}M) caused a significant transient relaxation in arteries with endothelium and no change in tension in arteries without endothelium (fig. 2). If the concentration of vasopressin in the organ bath was then doubled in a cumulative manner, after 3-4 minutes, no additional endothelium-dependent vasodilatory response could be elicited; only vasoconstriction was apparent (n=3, data not shown). The endothelium-dependent vasodilator response to vasopressin in arterial segments, contracted with prostaglandin $F_{2\alpha}$, could be inhibited by incubating the arterial segments with Ng-monomethyl-L-arginine (L-NMMA, 10^{-5}M) or N^G-nitro-L-arginine (L-NOARG, 10^{-4}M), two 2 inhibitors of nitric oxide synthesis from L-arginine (fig. 3). The inhibition of vasopressin-mediated vasorelaxation by L-NMMA could be reversed by addition of exogenous L-arginine (10^{-4} M) but not by D-arginine (10^{-4} M) (fig. 3).

Experiment 2 - Endothelium-dependent vasodilatation to vasopressin could also be inhibited by $d(CH_2)_5$ Try(Me) arginine vasopressin (10^{-6}M) (fig. 3). When quiescent coronary rings underwent this V_1 blocker action, any significant tension change was observed in vessels with and without endothelium (data not shown). The addition of $d(CH_2)_5$Try(Me) arginine vasopressin (10^{-6}M) caused no significant change in tension of quiescent coronary arterial segments with or without endothelium. When arterial segments that had been exposed to vasopressin were washed, contracted with prostaglandin $F_{2\alpha}$, and again exposed to the compound, transient endothelium-dependent relaxation was again observed (n=3, data not shown). The elapsed time between the 2 injections was the same 30-40 minutes, because indomethacin was added again to the coronary bath.

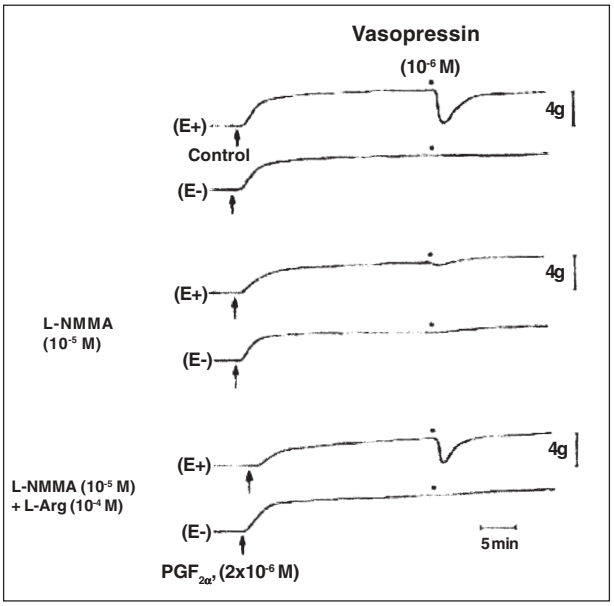

Fig. 2 - Isometric tension recording of the effect of arginine vasopressin on canine epicardial coronary arteries (original trace). Segments of left circumflex coronary artery, with and without endothelium, were suspended in organ chambers to measure isometric force. Segments were contracted with prostaglandin $F_{2\alpha}$ (2 x 10^{-6}M). When the contraction to prostaglandin was stable, the vessels were exposed to vasopressin (10^{-6}M)(top trace). Endothelium-dependent vasodilation to vasopressin could be inhibited by treating the vascular segments with L-NMMA (10^{-5}M)(middle trace). The inhibitory effect of L-NMMA could be reversed by addition of L-arginine (10^{-4}M)(bottom trace).

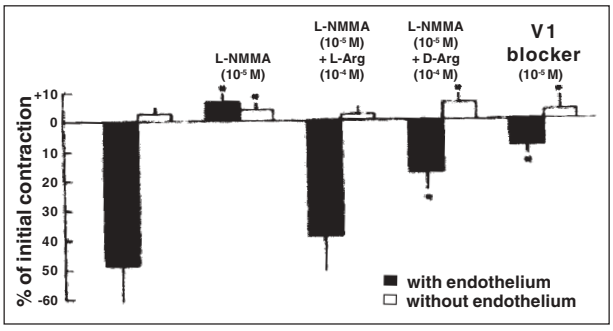

Fig. 3 - Effect of vasopressin in coronary arteries (Concentration-response bar graph). Segments were contracted with prostaglandin $F_{2\alpha}$ (2 x 10^{-6}M) and exposed to a single high concentration of vasopressin (10^{-6}M). Results are expressed as means ± SEM. L-NMMA denotes in the presence of N^G-monomethyl-L-arginine (10^{-5}M). L-NMMA + L-ARG denotes in the presence of N^G-monomethyl-L-arginine (10^{-5}M) and L-arginine (10^{-4}M). L-NMMA + D-ARG denotes in the presence of N^G-monomethyl-L-arginine (10^{-5}M) and D-arginine (10^{-4}M). V_1-blocker denotes in the presence of the vasopressin V_1-receptor antagonist $d(CH_2)_5$TYR (Me) AVP (10^{-6}M). Asterisk denotes significance from control (untreated) coronary artery segments with and without endothelium ($P<0.05$).

Experiment 3 - If vasopressin was added in a cumulative manner to the organ chamber, the endothelium-dependent relaxation to the compound was masked (fig. 4). Indeed, when the concentration of vasopressin was gradually increased in the organ bath, coronary artery segments with and without endothelium did not exhibit any significant difference between the injections of vasopressin. However, if, after being exposed to a high concentration of vasopressin (10^{-5}M), vascular segments were washed and immediately contracted with prostaglandin $F_{2\alpha}$, vasopressin (10^{-6}M)

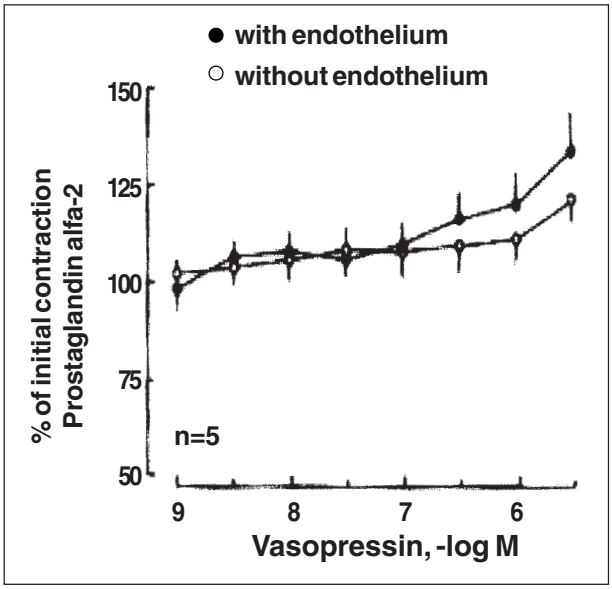

Fig. 4 - Concentration-response curves to vasopressin in canine coronary arteries (n=6). Segments were contracted with prostaglandin $F_{2\alpha}$ (2 x 10^{-6}M) and exposed to increasing concentrations of vasopressin. Results are expressed as means ± SEM.

caused transient, endothelium-dependent relaxation of arterial segments with endothelium. The complete concentration response curve lasted around 25 to 30 minutes, because the time observed among the increased concentration of vasopressin was around 3 minutes. The progressive increase in arterial tone was secondary to vasopressin action and was not dependent on the experimental time.

Discussion

It is relevant to include a comment about the nomenclature of the vasopressin receptors; if the prevalent rules were applied, the names of these receptors ought to be V_1 (V_1a), V_2 and V_3 (V_1b), with the V_1 and V_3 receptors modifying phospholipase activity and the V_2 receptor regulating adenylyl cyclase (AC) activity, as is the case for the muscarinic receptors. The meeting of seasoned investigators who must agree on the adoption of these rules has not yet taken place, and therefore the original names are still maintained; the change has been left for the new millennium [16].

Vasopressin effects on coronary arteries have already been reported in many species. In canine and cat coronary arteries, vasopressin elicits endothelium-dependent vasodilatation in large vessels and vasoconstriction in resistance vessels [1,17-19]. In other species (rabbit, rat, goat, and human), vasopressin induces a V_1 receptor-mediated vasoconstriction, eventually modulated by endothelial NO [20-24]. Pure vasodilatation seems to occur in isolated monkey coronary arteries via endothelium V_1 receptors and NO release [25].

In the present experiment, vasopressin induced transient, endothelium-dependent relaxation of the epicardial canine coronary artery, which was prevented by pretreating the vascular segments with Ng-monomethyl-L-arginine or NG-nitro-L-arginine, 2 inhibitors of nitric oxide synthesis from L-arginine [26,27]. The finding that the inhibition of vasodilatation by L-NMMA could be reversed by exogenous L-arginine emphasizes the specificity of the blockers for the L-arginine pathway [28]. This relaxation was also reversibly inhibited by $d(CH_2)_5$Try(Me) arginine vasopressin, suggesting that the vasodilator action is mediated by vasopressin V_1-receptors on the endothelium [29-31]. Thus, we conclude that vasopressin acts on endothelial cell V_1 receptors to stimulate the production of nitric oxide from L-arginine, and then mediates cyclic-GMP-dependent relaxation of the underlying vascular smooth muscle [32]. Similar conclusions were obtained in experiments performed with pulmonary canine arteries [33], monkey isolated coronary arteries [25] canine brain stem arteries [34], and in vivo experiments studying vasopressin in anesthetized goats [23].

When vasopressin was added in a cumulative manner starting at a very low concentration, the endothelium-dependent vasodilator effect was masked. Two possible speculative explanations for this observation exist. First, it is possible that desensitization of the V_1-receptor to activation by arginine vasopressin occurs at concentrations lower than the concentration necessary to induce EDNO production. Thus, when arginine vasopressin is given at a high concentration as a bolus injection, endothelial cell production of nitric oxide is stimulated to produce transient relaxation, but then the cell becomes refractory to further V_1-receptor stimulation. The finding supports this hypothesis that additional injections of arginine vasopressin did not relax the vessel after the initial transient vasodilatation. However, in contrast with this theory is the observation that if blood vessels, which had been exposed to high concentrations of vasopressin, were quickly washed, contracted with prostaglandin $F_{2\alpha}$ and exposed to vasopressin (10^{-6}M), they again exhibited transient, endothelium-dependent relaxation to the compound. One would not expect such a quick recovery of relaxation if the cell had truly become refractory to the compound. Indeed, by considering the modern concepts about the vasopressin receptors, it is impossible to rule out the role of desensitization. The ability of AVP to reduce the response of the V_1a and V_2 receptors was first described in liver and kidney cells, respectively, while characterizing the activity of the natural receptors; the availability of the cDNAs encoding the receptors made it possible to identify some biochemical details of this desensitization by the use of transfected cells. Desensitization of the V1aR is fast and is accompanied by sequestration of receptors inside the cell in tissues and transfected cells. AVP-promoted phosphorylation of the V1aR analyzed in transfected cells reached maximum values immediately after agonist binding, and the phosphates were removed rapidly from the protein with a t1/2 of 6 minutes, while disappearance of the receptor from the cell surface after exposure to AVP had a t1/2 of 3 minutes. After the removal of ligand from the medium, recycling of the V1aR to the cell surface was efficient and complete in about 60 minutes [16].

Another possibility is that the onset of the constrictor action of vasopressin comes before the stimulated production of EDNO. Indeed, if the vasoconstrictive action of vasopressin occurred at a concentration below that which induces EDNO formation, vasoconstriction would be expressed at low vasopressin concentrations and might completely mask the vasodilatory action of EDNO stimulated by higher concentrations of vasopressin. The finding supports this theory that the maximal contraction to vasopressin (10^{-5}M) was comparable in arterial segments with and without endothelium, an indication that vasopressin-mediated vasoconstriction can completely override cyclic GMP-mediated vasodilatation by vasopressin-stimulated EDNO production. Such a hypothesis is also consistent with studies demonstrating that the maximal tension developed to arginine vasopressin in the canine cerebral artery was unaffected by the presence or absence of an intact endothelium [35].

Interestingly, the vasorelaxation effect observed by using single vasopressin doses was no longer observed

when the concentration response curve to vasopressin was tested. A small vasoconstrictor effect appeared in this condition instead. Despite the fact that the present results are mostly confirmatory of the literature, it clearly shows the endothelium-dependent (likely NO-dependent) vasorelaxation induced by vasopressin in large coronary arteries. The NO release elicited by single doses and the loss of the concentration-related effect deserves more investigation. Perhaps this fact is related to some functional characteristic of the V1-receptor as above discussed [16].

Vasopressin-mediated production of EDNO in the coronary circulation could have physiological implications [4], by its selective vasoconstriction of regions with high O_2 supply/demand ratio serves to maintain vital tissue perfusion during periods of low cardiac output. If this were true, the stimulated production of EDNO by the coronary endothelium would also act to oppose ischemic events such as vasospasm and thrombosis through the anti-aggregatory [36] and vasodilatory action [37] of the nitric oxide radical.

Finally, it is important to emphasize that studies on vasopressin vascular effects would be helpful because of its growing importance in human cardiopulmonary resuscitation [38,39]. Coronary arteries probably never see such a high peptide 10^{-9} to 10^{-5} M concentration as used in this study. Physiological plasma levels are in the range of 10^{-12} to 10^{-11} M, which can increase to 10^{-9} M after dehydration, surgical stress, or in pathological conditions.

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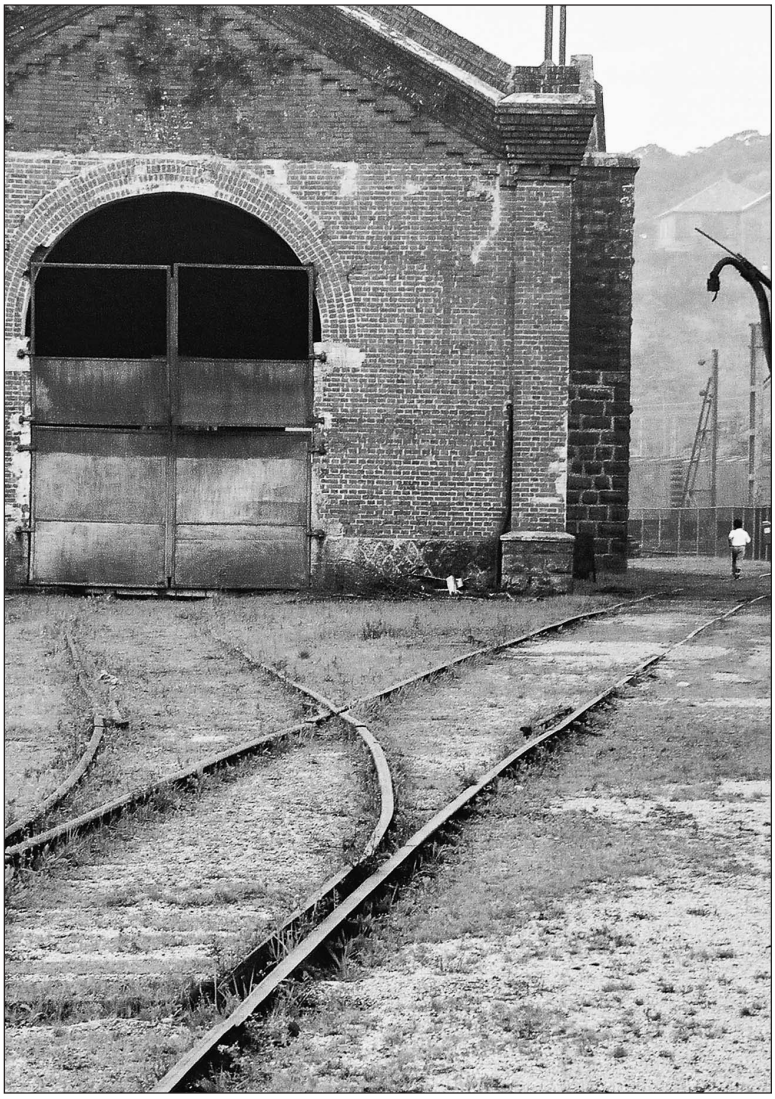

Paranapiacaba - SP *Dr. Mucio Tavares de Oliveira Jr - São Paulo*

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